

1                   **NOVEL CYTOTOXIC ANNONACEOUS ACETOGENINS FORM**

2                   ***ANNONA MURICATA***

3                   **ABSTRACT**

4               Acetogenins isolated from *Annona muricata* of the family Annonaceae are  
5               described. The substantially pure compounds of the invention exhibit to human tumor  
6               cell lines as well as selective cytotoxicity for various human tumor cell lines.

7                   **BACKGROUND OF THE INVENTION**

8               1. Field of the Invention

9               This invention relates to the isolation, identification and use of natural products  
10          as anti-tumor agents. More particularly, the present invention relates to seven new  
11          annonaceous acetogenins from *Annona muricata*, muricin A, B, C, D, E, F, and G, and  
12          their use in treating patients having tumors, and even some with hepatoma cancer.

213           2. Description of Related Art

14               Many plants of the Annonaceae have been used in folk medicine and  
15          insecticides. Among the constituents of these materials, annonaceous acetogenins,  
16          known to have potent anticancer activities, are regarded as the major active principles.  
17          Annonaceous acetogenins, a rather new class of natural compounds only isolated from  
18          the Annonaceae, are usually C<sub>35</sub>-C<sub>37</sub> fatty acid derivatives connecting a variable number  
19          of Tetrahydrofuran (THF) or Tetrahydropyran (THP) rings and lactone terminal moiety.  
20          So far, more than three hundred compounds, most of which were steric isomers, have  
21          been found and published, and their more biological activities, such as cytotoxic,  
22          antiparasitic, insecticide and immunosuppressive activities, have been further proved.

23               *Annona muricata* L. (Annonaceae) is a well-known tropical fruit tree named  
24          “sour sop” or “guanabana”, which is mainly distributed in the Americas and in Southeast

1 Asia. Currently, there have been more than forty annonaceous acetogenins isolated from  
2 the stems, leaves and seeds of this plant up. In the previous study of annonaceous  
3 acetogenins from *Annona muricata* by *Li's el al.*, three annonaceous acetogenins,  
4 muricatin A, muricatin B, and muricatin C, were found from the extract of the stem bark.  
5 In those annonaceous acetogenins, four known compounds, muricatetrocin A,  
6 muricatetrocin B, corossolin, and corosolone, show special selective cytotoxicities  
7 against hepatoma cell lines, Hep G<sub>2</sub>, and 2,2,15. These four compounds are discussed  
8 with seven newly discovered annonaceous acetogenins in the detailed description in  
9 cytotoxicities of curing hepatoma.

10 SUMMARY OF THE INVENTION

11 The main objective of the present invention is to disclose seven new  
12 annonaceous acetogenins, muricin A, B, C, D, E, F, and G, obtained from *Annona*  
13 *muricata*.

14 Another objective of the present invention is to disclose the curing features of  
15 eleven new and known annonaceous acetogenins in hepatoma cell lines.

16 Further benefits and advantages of the present invention will become apparent  
17 after a careful reading of the detailed description.

18 BRIEF DESCRIPTION OF THE FIGURES

19 Figure 1: The EI-MS Fragmentation of Muricin A (1) and Muricin B(2).

20 Figure 2: The EI-MS Fragmentation of Muricin C (3)

21 Figure 3: The EI-MS Fragmentation of Muricin D (4)

22 Figure 4: The EI-MS Fragmentation of Muricin E (5)

23 Figure 5: The EI-MS Fragmentation of Muricin F (6)

24 Figure 6: The EI-MS Fragmentation of Muricin G (7)

1    DETAILED DESCRIPTION OF THE INVENTION

2       This invention is directed to seven new Annonaceous acetogenins, muricin A (1),  
3       muricin B (2), muricin C (3), muricin D (4), muricin E (5), muricin F (6), and muricin G (7),  
4       isolated from *Annona muricata* in substantially pure form. As used herein, the term  
5       substantially pure form is defined as greater than 95% pure. In one embodiment, muricin A  
6       (1), B (2), C (3), D (4), E (5), F (6), and G (7), are isolated in greater than 99% pure form.  
7       Applicants have discovered that those seven compounds are cytotoxic to tumor cell lines,  
8       thus allowing their use for treating patients having a tumor.

9       In the present invention, these seven new annonaceous acetogenins, muricin A  
10      (1), B (2), C (3), D (4), E (5), F (6), and G (7), are disclosed in their chemical structures  
11      and chemical properties. These seven new annonaceous acetogenins are extracted from  
12      *A. muricata* seed as shown in the following experiment sections:

13      1. General experiment procedures:

14      Optical rotations were measured with a JASCO DIP-370 digital polarimeter.  
15      Melting points were determined using a Yanagimoto micro-melting point apparatus and  
16      were uncorrected. The IR spectra were measured on a Hitachi 260-30 spectrophotometer.  
17       $^1\text{H}$  NMR (400MHz) and  $^{13}\text{C}$  NMR (100 MHz) spectra in  $\text{CDCl}_3$  were recorded with  
18      Varian NMR spectrometers, using TMS as the internal standard. LRFABMS and  
19      LREIMS were obtained with a JOEL JMS-SX/SX 102A mass spectrometer or a Quattro  
20      GC/MS spectrometer having a direct inlet system. HRFABMS were measured in a JEOL  
21      JMS-HX 110 mass spectrometer. CD was measured on a JASCO DIP 370 polarimeter.  
22      Si gel 60 (Macherey-Nagel, 230-400 mesh) was used for column chromatography;  
23      precoated Si gel plates (Macherey-Nagel, SIL G-25 UV<sub>254</sub>, 0.25mm) were used for  
24      analytical TLC, and precoated Si gel plates (Macherey-Nagel, SIL G/UV<sub>254</sub>, 0.25 mm)

1 were used for the preparative TLC. The spots were detected by spraying with  
2 Dragendorff's reagent or 50% H<sub>2</sub>SO<sub>4</sub> and then heating on a hot plate. HPLC was  
3 performed on a JASCO PU-980 apparatus equipped with a UV-970 detector. Develosil  
4 ODS-5 (250 x 4.6mm i.d.) and preparative ODS-5 (250 x 20mm i.d.) columns were used  
5 for analytical and preparative purposes, respectively.

6       2. Plant material:

7           The seeds of *Annona muricata* were collected from Chia-Yi City, Taiwan,  
8 Republic of China, in March 1997. A voucher specimen is deposited in the Graduate  
9 Institute of Natural Products, Kaohsiung, Taiwan, Republic of China.

10          3. Extraction and isolation:

11           The seeds (1.0 kg) were extracted repeatedly with MeOH at room temperature.  
12 The combined MeOH extracts were evaporated and partitioned to yield CHCl<sub>3</sub> and  
13 aqueous extracts. The CHCl<sub>3</sub> layer afforded a waxy extract (*ca.* 200.6 g), positive to  
14 Kedde's reagent. The CHCl<sub>3</sub> layer was further separated into ten fractions by column  
15 chromatography on Si gel with gradient system of *n*-hexane-CHCl<sub>3</sub> (*n*-hexane-CHCl<sub>3</sub>  
16 4:1 to pure CHCl<sub>3</sub>) and CHCl<sub>3</sub>-MeOH (pure CHCl<sub>3</sub> to CHCl<sub>3</sub>-MeOH: 10:1).  
17 Longifolicin, corossolin, and corossolone were further purified from the eighth fraction  
18 by reversed-phase HPLC. Then, the remnant of the eighth fraction was combined with  
19 the ninth fraction and further separated into ten fractions by column chromatography  
20 with reversed-phase HPLC. Muricin A (1), muricin B (2), muricin C (3), and muricin F  
21 (6) were isolated and purified from the seventh fraction by a preparative reversed-phase  
22 HPLC (ODS-5 column) with 88:12 MeOH-water (flow rate of 2 mL/min; UV detector  
23 set at 225nm). Muricin D (4), muricin E (5), and muricin G (7), as well as two known  
24 compounds, muricatetrocin A (8) and muricatetrocin B (9), were isolated and purified

1 from the eighth fraction by a preparative reversed-phase HPLC with 86:14 MeOH-water  
2 (flow rate of 2 mL/min; UV detector set at 225nm).

3 Annonaceous acetogenins compounds 1-9 were further studied in their molecular  
4 structure as following:

5 1.Muricin A (1) was obtained as a white waxy solid;  $[\alpha]^{25}_D +7.2^0$  (c 0.25,  $\text{CHCl}_3$ );  
6 UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \varepsilon$ ) 210 (3.64) nm ; IR (KBr)  $\nu_{\text{max}}$  3392 (OH), 2917, 2849, 1746  
7 ( $\text{OC=O}$ ), 1067  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100MHz) ;  
8 FABMS  $m/z$  597[ $\text{M+H}^+$ ] ; EIMS (30eV) 381 (2), 363 (1), 351 (11), 333 (11), 281 (30), 263  
9 (2), 239 (19), and 221 (5), see figure 1 ; HRFABMS  $m/z$  597.4726 (calcd. For  $\text{C}_{35}\text{H}_{65}\text{O}_7$ ,  
10 597.4730).

11 The  $[\text{M+Na}]^+$  peak in the FABMS at  $619m/z$  established the molecule weight as 596.  
12 The HRFABMS gave an  $[\text{M+H}]^+$  peak at  $m/z$  597.4726 (calcd. 597.4730), corresponding  
13 to the molecular formula,  $\text{C}_{35}\text{H}_{65}\text{O}_7$ . The UV spectral absorption at 210nm and the IR  
14 spectral absorption at  $1740\text{cm}^{-1}$  indicated the presence of an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone  
15 group, positive to Kedde's reagent. The successive EI-MS peaks at  $m/z$  578, 560, and 542  
16 implied the appearance of three hydroxyl groups at least. In the  $^1\text{H-NMR}$  spectrum, the  
17 signals at  $\delta$  7.18 (1H, H-33), 5.06 (1H, H-34), 3.81 (1H, H-4), 2.54 (1H, H-3a), 2.47 (1H,  
18 H-3b), and 1.41 (3H, H-35) verified the presence of an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a  
19 hydroxyl group at C-4 position (see Table 1).

20

21 Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$  NMR Chemical Shifts of Compounds 1 and 2

	Muricin A		Muricin B	
	$\delta$ ( $^1\text{H}$ )	$\delta$ ( $^{13}\text{C}$ )	$\delta$ ( $^1\text{H}$ )	$\delta$ ( $^{13}\text{C}$ )
1		174.9		174.6

2		131.2		131.9
3a	2.54 (m)	32.2	2.53 (m)	32.4
3b	2.47 (m)		2.49 (m)	
4	3.81 (m)	69.8	3.79 (m)	69.8
5	1.2~1.5	37.1	1.2~1.5	37.2
6~13	1.2~1.5	25.3-29.8	1.2~1.5	25.5-30.4
14	1.2~1.5	35.3	1.2~1.5	35.4
15	3.81 (m)	79.3	3.79 (m)	79.3
16	1.97, 1.62 (m)	25.3-29.8	1.98, 1.63 (m)	25.5-30.4
17	1.97, 1.62 (m)	25.3-29.8	1.98, 1.63 (m)	25.5-30.4
18	3.81 (m)	81.8	3.79 (m)	81.8
19	3.41 (m)	74.4	3.40 (m)	74.5
20	1.5~1.6	33.1-33.3	1.5~1.6	33.3-33.7
21~24	1.2~1.5	25.3-29.8	1.2~1.5	25.5-30.4
25	1.2~1.5	33.1-33.3	1.2~1.5	33.3-33.7
26	3.41 (m)	74.9 <sup>a</sup>	3.40 (m)	74.7 <sup>a</sup>
27	3.41 (m)	74.2 <sup>a</sup>	3.40 (m)	74.3 <sup>a</sup>
28	1.2~1.5	33.1-33.3	1.2~1.5	33.3-33.7
29	1.2~1.5	25.3-29.8	1.2~1.5	25.5-30.4
30	1.2~1.5	31.8	1.2~1.5	31.9
31	1.2~1.5	22.5	1.2~1.5	22.7
32	0.86 (t, <i>J</i> =6.7)	13.9	0.87 (t, <i>J</i> =6.7)	14.1
33	7.18 (d, <i>J</i> =1.6)	152.1	7.18 (d, <i>J</i> =1.2)	151.9
34	5.06 (qd, <i>J</i> =6.8, 1.6)	78.0	5.06 (qd, <i>J</i> =6.4, 1.2)	78.0

35	1.41 (d, $J=6.8$ )	18.9	1.41 (d, $J=6.8$ )	19.1
----	--------------------	------	--------------------	------

1       <sup>a</sup>Assignments may be interchangeable.

2  
3       The signal at  $\delta$  3.81 (2H, H-15, 18), 3.41 (1H, H-19), as well as  $^{13}\text{C}$ -NMR peaks at  
4        $\delta$  81.8 (C-18), 79.3 (C-15), 74.4 (C-19), indicated the presence of a mono-THF ring with  
5       one flanking hydroxyl in a *threo*-conformation. A close examination of the NMR spectrum  
6       showed the proton resonances for the two methylene groups of the mono-THF ring, which  
7       were observed at  $\delta$  1.97 (H-16a, 17a) and 1.62 (H-16b, 17b), were corresponding to the  
8       *trans* conformation. By making the (*R*) and (*S*)-Mosher ester derivatives and Hoey  
9       methodology, the absolute stereochemistries at C-4 and C-19 of the muricin A could be  
10      confirmed as (*R*) and (*R*) respectively (see Table 2).

11  
12      Table 2.  $^1\text{H}$  NMR Data of the (*S*)- and (*R*)-Mosher Esters of **1** and **2**

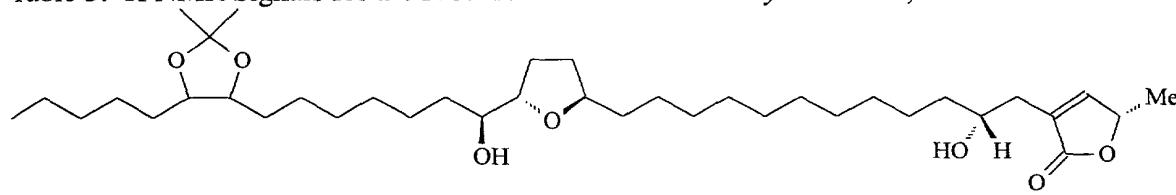
Protons	<b>1</b>				<b>2</b>			
	<i>S</i> -MTPA	<i>R</i> -MTPA	$\Delta \delta_{S-R}$	Config	<i>S</i> -MTPA	<i>R</i> -MTPA	$\Delta \delta_{S-R}$	config
H-3a	2.67	2.68	-0.01		2.63	2.53	+0.01	
H-3b	2.58	2.59	-0.01		2.57	2.40	+0.17	
H-33	6.96	6.96	0		6.72	6.65	+0.07	
H-34	4.89	4.90	-0.01	4 <i>R</i>	5.09	5.05	+0.04	4 <i>S</i>
H-15	3.87	3.88	-0.01		3.85	3.88	-0.10	
H-18	3.71	3.72	-0.01	19 <i>R</i>	3.80	3.82	-0.02	19 <i>R</i>

13  
14      The two of these hydroxyl groups were suspected as the presence of a vicinal diol due  
15      to the proton signal at  $\delta$  3.41 (2H) and  $^{13}\text{C}$  NMR peaks at  $\delta$  74.9 and 74.2. By making the

1 acetonide derivative, the downfield shifts of two protons from  $\delta$  3.41 to 3.60 for two of the  
2 three methylene protons on OH-bearing carbons and the chemical shift of six protons at  $\delta$   
3 1.36 ( $2 \times \text{CH}_3$ , s) in the  $^1\text{H}$ -NMR spectrum verified the presence of one vicinal diol (see  
4 Table 3). The conformation of this vicinal diol was assigned as *threo* based on a  
5 comparison of its NMR chemical shift with literature data.

6

7 Table 3.  $^1\text{H}$  NMR Signals for the Protons of the *Threo* and *Erythro* Diols,<sup>13</sup> **1** and Acetonide **1b**



	Methine protons		Acetonyl methyls	
	<i>threo</i>	<i>erythro</i>	<i>Threo</i>	<i>erythro</i>
Diol	3.45 (2H)	3.62, 3.58		
Acetonide	3.58 (2H)	4.02, 4.00	1.37 (6H)	1.43, 1.33
<b>1</b>	3.41 (2H)			
<b>1b</b>	3.60 (2H)		1.36 (6H)	

9

10 The placement of the THF ring and the diol were established by close examination of  
11 EI-MS fragmentation of muricin A (see figure 1). The THF ring was placed between C-15  
12 and C-18 based on the EI-MS fragmentation at *m/z* 351 and 281, and the vicinal diol was  
13 located at C-26/C-27 based on the EI-MS fragments at *m/z* ([711]→[621]→531→441) of  
14 the TMS derivative.

15 Finally, the absolute configuration at C-34 of muricin A was determined by the CD  
16 method. According to a positive  $\pi - \pi^*$  Cotton effect ( $\Delta \varepsilon > 0$ ), it clearly indicated that the  
17 stereochemistry at C-34 on the  $\gamma$ -lactone fragment should be (S)-configuration.

1        2. Muricin B (2) was obtained as a white waxy solid;  $[\alpha]^{25}_{\text{D}} +0.2^0$  (c 0.11,  $\text{CHCl}_3$ );  
2        UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \varepsilon$ ) 210 (3.65) nm ; IR (KBr)  $\nu_{\text{max}}$  3419 (OH), 2918, 2849, 1738  
3        ( $\text{OC=O}$ ), 1067  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) data ;  
4        FABMS  $m/z$  597[M+H] $^+$  ; EIMS (30eV) 381 (2), 363 (1), 351 (10), 333 (11), 281 (30), 263  
5        (2), 239 (19), 221 (5), see figure 1 ; HRFABMS  $m/z$  597.4731 (calcd. for  $\text{C}_{35}\text{H}_{65}\text{O}_7$ ,  
6        597.4730).

7        Muricin B was separated and afforded following muricin A by reversed-phase HPLC  
8        with a solvent system MeOH/ $\text{H}_2\text{O}$  (88/12), and muricin A and muricin B showed the  
9        completely different retention time at 13.7min and 14.2min, respectively (Develosil  
10      ODS-5 column, 250x4.6 mm i.d., flow rate of 1 mL/min). The HRFAB-MS gave an  
11      [M+H] $^+$  peak at  $m/z$  597.4731 (calcd. 597.4730), corresponding to the molecular formula,  
12       $\text{C}_{35}\text{H}_{65}\text{O}_7$ . Interestingly, from comparisons with the NMR spectral and MS data of muricin  
13      A, it was clearly indicated that muricin B had high similarity to muricin A. Like muricin A,  
14      the  $^1\text{H}$  and  $^{13}\text{C}$ -NMR signals indicated the presence of an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone  
15      with a hydroxyl group at C-4 position and a mono THF ring with one flanking hydroxyl  
16      group in a relative conformation of *trans/threo* according to Fujimoto *et al.* (see Table 1).  
17      The normal-form tail of muricin B was corroborated by the absorptions in the IR at  
18      1740  $\text{cm}^{-1}$  and UV  $\lambda_{\text{max}}$  at 210nm. The vicinal diol was confirmed by making its acetonide  
19      derivative and determined its conformation as *threo* based in the comparison of the  $^{13}\text{C}$   
20      NMR and  $^1\text{H}$ -NMR data with muricin A.

21        The EI-MS data of muricin B similar to one of muricin A determined the placement of  
22        the THF ring and the diol at C-15/C-18 and C-26/C-27, respectively (see figure 1). The  
23        positive  $\pi - \pi^*$  Cotton effect ( $\Delta \varepsilon > 0$ ) of muricin B in CD spectrum indicated the  
24        stereochemistry at C-34 on the  $\gamma$ -lactone fragment be (S)-configuration.

1       3. Muricin C (3) was obtained as a white waxy solid;  $[\alpha]^{25}_{\text{D}} +86.0^{\circ}$  (*c* 0.15,  $\text{CHCl}_3$ );  
2   UV (MeOH)  $\lambda_{\text{max}}$  ( $\log \varepsilon$ ) 208 (3.73) nm ; IR (KBr)  $\nu_{\text{max}}$  3440 (OH), 2930, 2833, 1745  
3   ( $\text{OC=O}$ ), 1027  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) data;  
4   FABMS *m/z* 597 [ $\text{M}+\text{H}]^+$ ; EIMS (30eV) 449 (1), 431 (1), 409 (1), 391 (1), 379 (6), 361 (9),  
5   309 (16), 291 (3), 267 (10), 239 (5), see figure 2; HRFABMS *m/z* 597.4732 (calcd. for  
6    $\text{C}_{35}\text{H}_{65}\text{O}_7$ , 597.4730).

7       The HRFAB-MS gave an  $[\text{M}+\text{H}]^+$  peak at *m/z* 597.4726 (calcd. 597.4730),  
8   corresponding to the molecular formula,  $\text{C}_{35}\text{H}_{65}\text{O}_7$ . The successive FAB-MS fragment at  
9   *m/z* 579, 561, 543, 525 suggested the presence of four hydroxyl groups. The UV absorption  
10   at 208nm and the IR absorption at 1740 $\text{cm}^{-1}$  indicated the presence of an  $\alpha, \beta$ -unsaturated  
11    $\gamma$ -lactone with a hydroxyl group at C-4, a mono-THF ring with one flanking hydroxyl  
12   group, and a vicinal diol.

13       The signals of  $^1\text{H}$ -NMR spectrum at  $\delta$  7.18 (1H, H-33), 5.06 (1H, H-34), 3.86 (1H,  
14   H-4), 2.52 (1H, H-3a), 2.40 (1H, H-3b), 1.42 (3H, H-35), together with  $^{13}\text{C}$ -NMR peaks at  
15    $\delta$  174.6 (C-1), 151.8 (C-33), 131.2 (C-2), 78.0 (C-34), 70.0 (C-4), and 19.1 (C-35), were  
16   matched well the published data of an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group  
17   at C-4. The proton signals at  $\delta$  3.86 (2H, H-17, 20), 3.43 (1H, H-21), 1.97 (2H, H-18a, 19a),  
18   1.65 (2H, 18b, 19b), as well as  $^{13}\text{C}$ -NMR peaks at  $\delta$  81.7 (C-20), 79.3 (C-17), and 74.4  
19   (C-21), indicated the presence of a mono-THF ring with one flanking hydroxyl in relative  
20   of *trans/threo* or *threo/trans* conformation. Like muricin A, two of the four hydroxyl  
21   groups were determined as a diol due to the proton signal at  $\delta$  3.43(2H) and  $^{13}\text{C}$ -NMR  
22   peaks at  $\delta$  74.6 & 74.3 (see Table 4).

23       The placements of the THF ring and hydroxyl groups were established by close  
24   examination of EI-MS fragmentation of muricin C (see figure 2). The THF ring was

1 located between C-17/C-20 based on the EI-MS peaks at *m/z* 379 and 309, and the vicinal  
2 diol was placed between C-24/C-25 according to the EI-MS peaks at *m/z* ([467]→449→  
3 431).

4 Finally, the absolute configuration at C-34 of muricin C was determined by the CD  
5 method. The positive  $\pi - \pi^*$  Cotton effect ( $\Delta \varepsilon > 0$ ) clearly indicated the stereochemistry at  
6 the C-34 on the  $\gamma$ -lactone fragment was (S)-configuration. Additionally, muricin C is also  
7 the first example of annonaceous acetogenins that the THF ring began with an odd position  
8 C-17.

9 4. Muricin D (4) was obtained as white waxy solid;  $[\alpha]^{25}_D +77.6^0$  (c 0.34,  $\text{CHCl}_3$ );  
10 UV (MeOH)  $\lambda_{\max}$  ( $\log \varepsilon$ ) 208 (3.69) nm ; IR (KBr)  $\nu_{\max}$  3432 (OH), 2925, 2854, 1745  
11 (OC=O), 1462, 1319, 1082  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ,  
12 100MHz) data; FABMS *m/z* 569 [ $\text{M}+\text{H}]^+$  ; EIMS (30eV) 439 (1), 421 (1), 403 (1), 381 (2),  
13 363 (1), 351 (30), 333 (21), 281 (64), 263 (4), 239 (40), 221 (7), see figure 3; HRFABMS  
14 *m/z* 569.4416 (calcd. for  $\text{C}_{33}\text{H}_{60}\text{O}_7$ , 569.4417).

15 The HRFAB-MS gave an  $[\text{M}+\text{H}]^+$  peak at *m/z* 569.4416 (calcd. 569.4417),  
16 corresponding to the molecular formula  $\text{C}_{33}\text{H}_{61}\text{O}_7$ . The successive FAB-MS fragment at  
17 *m/z* 551, 533, 515 , 497 suggested the presence of four hydroxyl groups. The IR absorption  
18 at 1740 $\text{cm}^{-1}$  and the UV absorption at 208nm indicated the presence of a  $\gamma$ -lactone group.  
19 Comparisons with the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data of muricin A and muricin C  
20 suggested that muricin D also have the same moieties, an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone  
21 with a hydroxyl group at C-4, a mono-THF ring diol with a conformation of *threo*  
22 according to the method of Fujimoto *et al.* (see Table 4).

23 The structure of the molecule was established by close examination of EI-MS  
24 fragmentation of muricin D (see figure 3). The fragments at *m/z* 351 and 281 indicated that

1 the THF ring be located between C-15/C-18, and the fragments at *m/z* (439→421)  
2 indicated the diol be located between C-22/C-23.

3 5. Muricin E (5) was obtained as a white waxy solid;  $[\alpha]^{25}_D +91.4^0$  (*c* 0.23,  $\text{CHCl}_3$ );  
4 UV (MeOH)  $\lambda_{\max}$  ( $\log \varepsilon$ ) 208 (3.62) nm ; IR (KBr)  $\nu_{\max}$  3334 (OH), 2916, 2847, 1733  
5 ( $\text{OC=O}$ ), 1082  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) data;  
6 FABMS *m/z* 569[M+H] $^+$ ; EIMS (30eV) 421 (1), 403 (1), 351 (7), 333 (10), 309 (4), 291 (2),  
7 263 (4), 239 (24) see figure 4 ; HRFABMS *m/z* 569.4417 (calcd. for  $\text{C}_{33}\text{H}_{60}\text{O}_7$ , 569.4417).

8 The HRFAB-MS gave an [M+H] $^+$  peak at *m/z* 569.4417 (calcd. 569.4417),  
9 corresponding to the molecular formula  $\text{C}_{33}\text{H}_{61}\text{O}_7$ . The successive FAB-MS fragments at  
10 *m/z* 551, 533, 515, and 497, suggested the presence of four hydroxyl groups. The IR  
11 absorption at 1740  $\text{cm}^{-1}$  and the UV absorption at 208nm indicate the presence of a  $\gamma$ -  
12 lactone group. Comparisons with the NMR spectral data of muricin A and muricin C  
13 suggested that muricin E possesses the same moieties, an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone  
14 with a hydroxyl group at C-4, a mono-THF ring with one flanking hydroxyl group in a  
15 conformation of *threo/trans*, and a vicinal diol with a conformation of *threo* according to  
16 the method of Fujimoto *et al* (see Table 4).

17 The structure of the molecule was established by close examination of EI-MS  
18 fragmentation of muricin E (see figure 4). The fragment at *m/z* 309 and 239 demonstrated  
19 that the THF ring should be located between C-12/C-15, and the EI-MS fragments at *m/z*  
20 ([439]→421→403) indicated the diol should be located between C-22/C-23.

21 6. Muricin F (6) was obtained as a white waxy solid;  $[\alpha]^{25}_D +48.2^0$  (*c* 0.48,  $\text{CHCl}_3$ );  
22 UV (MeOH)  $\lambda_{\max}$  ( $\log \varepsilon$ ) 208 (3.89) nm ; IR (KBr)  $\nu_{\max}$  3407 (OH), 2925, 2854, 1743  
23 ( $\text{OC=O}$ ), 1078  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) data;  
24 FABMS *m/z* 595 [M+H] $^+$ ; EIMS (30eV) 507 (1), 477 (1), 459 (1), 423 (1), 405 (1), 379 (2),

1 361 (2), 309 (7), 281 (15), 263 (3), 239 (12), 109 (13), see figure 5; HRFABMS *m/z*  
2 595.4573 (calcd. for C<sub>35</sub>H<sub>62</sub>O<sub>7</sub>, 595.4574).

3 The HRFAB-MS gave an [M+H]<sup>+</sup> peak at *m/z* 595.4573 (calcd. 595.4574),  
4 corresponding to the molecular formula C<sub>33</sub>H<sub>61</sub>O<sub>7</sub>. The UV absorption at 208nm and the IR  
5 absorption at 1743cm<sup>-1</sup> indicated the presence of an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone group.  
6 Comparisons with the NMR spectral data of muricin A and muricin F suggested that  
7 muricin F have the same moieties, an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group  
8 in a conformation of *threo/trans*, and a vicinal diol with a conformation of *threo* according  
9 to the methods of Fujimoto *et al*(see Table 5). Moreover, the proton signal at  $\delta$  5.39 (2H),  
10 together with <sup>13</sup>C-NMR peaks at  $\delta$  130.1 and 129.5, showed the presence of a double bond.

11  
12 Table 5. <sup>1</sup>H- and <sup>13</sup>C NMR Chemical Shifts of Compounds **6** and **7**

	Muricin F		Muricin G	
	$\delta$ ( <sup>1</sup> H)	$\delta$ ( <sup>13</sup> C)	$\delta$ ( <sup>1</sup> H)	$\delta$ ( <sup>13</sup> C)
1		174.6		174.6
2		131.1		131.1
3a	2.52 (m)	32.4	2.50 (m)	33.3-33.4
3b	2.40 (m)		2.41 (m)	
4	3.80 (m)	69.9	3.82 (m)	69.9
5	1.2~1.5	37.4	1.2~1.5	37.3
6-8	1.2~1.5	25.5-29.9	1.2~1.5	25.5-29.9
9	1.2~1.5	25.5-29.9	1.2~1.5	33.3-33.4
10	1.2~1.5	25.5-29.9	3.58	71.7
11	1.2~1.5	25.5-29.9	1.2~1.5	33.3-33.4

12-13	1.2~1.5	25.5-29.9	1.2~1.5	25.5-29.9
14	1.2~1.5	25.5-29.9	1.2~1.5	37.2
15	1.2~1.5	25.5-29.9	3.43 (m)	74.0
16	1.2~1.5	33.1-35.4	3.89 (m)	82.6 <sup>a</sup>
17	3.86 (m)	79.3	1.99, 1.65 (m)	25.5-29.9
18	1.97, 1.65 (m)	25.5-29.9	1.99, 1.65 (m)	25.5-29.9
19	1.97, 1.65 (m)	25.5-29.9	3.89 (m)	82.6 <sup>a</sup>
20	3.86 (m)	81.7	3.43 (m)	73.5
21	3.43 (m)	74.4 <sup>a</sup>	1.2~1.5	33.3-33.4
22	1.5~1.6	33.1-35.4	2.17 (m)	25.5-29.9
23	2.01	25.5-29.9	5.36 (m)	130.8
24	5.39 (m)	130.1	5.36 (m)	128.9
25	5.39 (m)	129.5	2.17 (m)	25.5-29.9
26	2.01	33.1-35.4	1.2~1.5	25.5-29.9
27	3.42 (m)	74.6 <sup>a</sup>	1.2~1.5	25.5-29.9
28	3.42 (m)	74.3 <sup>a</sup>	1.2~1.5	25.5-29.9
29	1.2~1.5	33.1-35.4	1.2~1.5	25.5-29.9
30	1.2~1.5	31.4	1.2~1.5	31.9
31	1.2~1.5	22.6	1.2~1.5	22.7
32	0.87 (t, J=6.7)	14.0	0.87 (t, J=6.8)	14.1
33	7.18 (d, J=1.2)	151.9	7.18 (d, J=1.2)	151.9
34	5.06 (qd, J=6.8, 1.2)	78.0	5.05(qd, J=6.8, 1.2)	78.0
35	1.42 (d, J=6.8)	19.1	1.41(d, J=6.8)	19.1

1       The structure of the molecule was established by close examination of EI-MS  
2 fragmentation of muricin F (see figure 5). The EI-MS peaks of muricin F at *m/z* 379 and  
3 309 demonstrated that the THF ring should be located between C-17/C-20. Furthermore,  
4 the position of the double bond was determined at C-24/C-25 based on the EI-MS peak at  
5 *m/z* 477 and 423. Finally, the EI-MS peak at *m/z* 507 indicated that the diol should be  
6 located between C-27/C-28.

7       7. Muricin G (7) was obtained as a white waxy solid;  $[\alpha]^{25}_D +47.0^0$  (*c* 0.63,  $\text{CHCl}_3$ );  
8 UV (MeOH)  $\lambda_{\max}$  ( $\log \epsilon$ ) 210 (3.52) nm; IR (KBr)  $\nu_{\max}$  3386 (OH), 2931, 2859, 1748  
9 ( $\text{OC=O}$ ), 1081  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) data;  
10 FABMS *m/z* 595 [ $\text{M+H}]^+$ ; EIMS (30eV) 495 (1), 423 (1), 397 (1), 379 (2), 361 (6), 309  
11 (44), 291 (15), 273 (7), 241 (13) see figure 6; HRFABMS *m/z* 595.4574 (calcd. for  
12  $\text{C}_{35}\text{H}_{62}\text{O}_7$ , 595.4574).

13       The HRFABMS gave an  $[\text{M+H}]^+$  peak at *m/z* 595.4574 (calcd. 595.4574),  
14 corresponding to the molecular formula,  $\text{C}_{35}\text{H}_{62}\text{O}_7$ . The UV absorption at 210nm and IR  
15 absorption at 1748  $\text{cm}^{-1}$  indicated the presence of an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone group. In  
16 comparison with the NMR spectral data of muricin A and the published data of asiminenin  
17 B, Woo *et al*, *Heterocycles*, 41, 1731-1742 (1995), it was clearly suggested that muricin G  
18 has a high similarity to asiminenin B (see Table 5).

19       The proton signals at  $\delta$  7.18 (1H, H-33), 5.05 (1H, H-34), 3.89 (1H, H-4), 2.50 (1H,  
20 H-3a), 2.41 (1H, H-3b), 1.41 (3H, H-35) together with  $^{13}\text{C}$ -NMR peaks at  $\delta$  174.6 (C-1),  
21 151.9 (C-33), 131.1 (C-2), 78.0(C-34), 69.9 (C-4), 19.1 (C-35), were matched well with  
22 the published data of an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group at C-4  
23 position. The proton signals at  $\delta$  3.89 (2H, H-16, 19), 3.43 (2H, H-15, 20), 1.99 (2H, H-  
24 16a, 17a), 1.65 (2H, H-16b, 17b), as well as  $^{13}\text{C}$ -NMR peaks at  $\delta$  82.6 (C-18), 74.0 (C-15),

1 and 73.5(C-19), indicated the presence of a mono-THF ring with two flanking hydroxyl  
2 groups in the conformation of *threo/trans/threo* according to the method of Fujimoto *et al.*

3 The structure of the molecule was established by close examination of EI-MS  
4 fragment of muricin G (see figure 6). The EI-MS fragment at *m/z* 241 (cleavage between  
5 C-10/C-11) and its daughter peak at *m/z* 223 (cleavage between C-10/C-11--H<sub>2</sub>O)  
6 suggested that the final hydroxyl group should be located at C-10. The EI-MS peaks at *m/z*  
7 397 and 309 demonstrated the location of the THF ring should be between C-16/C-19.  
8 Finally, the peak at *m/z* 495 suggested that the double bond should be located at C-23/C-24.

9 All the CD spectra of compounds 4-7 were shown the positive  $\pi - \pi^*$  Cotton effect  
10 ( $\Delta \varepsilon > 0$ ), which indicated the stereochemistry at the C-34 on the  $\gamma$ -lactone fragment  
11 should be (S)-configuration as the same as muricin A, muricin B and muricin C.

12 8. A mixture of muricatetrocin A (8) and muricatetrocin B (9) was obtained as a  
13 colorless oil;  $[\alpha]^{25}_D +22.2^0$  (c 0.25, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max} (\log \varepsilon)$  210 (3.94) nm; UV,  
14 MS, <sup>1</sup>H- and <sup>13</sup>C-NMR data were identical with published values (see reference 5).

15 9. Longifolicin (10) was obtained as a colorless oil;  $[\alpha]^{25}_D +8.3^0$  (c 0.12, CHCl<sub>3</sub>); UV  
16 (MeOH)  $\lambda_{\max} (\log \varepsilon)$  208 (3.98) nm; UV, MS, <sup>1</sup>H- and <sup>13</sup>C-NMR were identical with  
17 published values. (see reference 6)

18 10. Corossolin (11) was obtained as a waxy solid;  $[\alpha]^{25}_D +82.8^0$  (c 0.34, CHCl<sub>3</sub>); UV  
19 (MeOH)  $\lambda_{\max} (\log \varepsilon)$  210 (3.78) nm; UV, MS, <sup>1</sup>H- and <sup>13</sup>C-NMR were identical with  
20 published values. (see reference 7)

21 11. Corossolone (12) was obtained as a waxy solid;  $[\alpha]^{25}_D +11.7^0$  (c 0.19, CHCl<sub>3</sub>);  
22 UV (MeOH)  $\lambda_{\max} (\log \varepsilon)$  206 (3.98) nm; UV, MS, <sup>1</sup>H- and <sup>13</sup>C-NMR were identical with  
23 published values. (see reference 7)

24

1        One embodiment of the present invention provides pharmaceutical formulations  
2        comprising an effective amount of muricins A-G (1-7) for treating a patient having a tumor.  
3        As used herein, an effective amount of the acetogenin compound is defined as the amount  
4        of the compound that, upon administration to a patient, inhibits growth of tumor cells, kills  
5        malignant cells, reduces the volume or size of the tumors or eliminates the tumor entirely in  
6        the treated patient. Thus, the substantially pure compounds in accordance with this  
7        invention can be formulated into dosage forms using pharmaceutically acceptable carriers  
8        for oral or parenteral administration to patients in need of oncolytic therapy. In one  
9        embodiment, a chemotherapeutic composition comprises an anti-tumor effective amount  
10      of a compound selected from the group consisting of muricins A-G (1-7) and a  
11      pharmaceutically acceptable carrier.

12       Effective doses will also vary, as recognized by those skilled in the art, dependant on  
13      route of administration, excipient usage and the possibility of co-usage with other  
14      therapeutic treatments including other anti-tumor agents, and radiation therapy.

15       The present pharmaceutical formulation may be administered via the parenteral route,  
16      including subcutaneously, intraperitoneally, intramuscularly and intravenously. Examples  
17      of parenteral dosage forms include aqueous solutions of the active agent, in an isotonic  
18      saline, 5% glucose or other well-known pharmaceutically acceptable liquid carrier. In one  
19      preferred aspect of the present embodiment, the acetogenin compound is dissolved in a  
20      saline solution containing 5% of dimethyl sulfoxide and 10% Cremphor EL (Sigma  
21      Chemical Company). Additional solubilizing agents such as cyclodextrins, which form  
22      specific, more soluble complexes with the present acetogenin compounds, or other  
23      solubilizing agents well-known to those familiar with the art, can be utilized as  
24      pharmaceutical excipients for delivery of the acetogenin compounds. Alternatively, the

1 present compounds can be chemically modified to enhance water solubility.  
2 The present compounds can also be formulated into dosage forms for other routes of  
3 administration utilizing well-known methods. The pharmaceutical compositions can be  
4 formulated, for example, in dosage forms for oral administration in a capsule, a gel seal or  
5 a tablet. Capsules may comprise any well-known pharmaceutically acceptable material  
6 such as gelatin or cellulose derivatives. Tablets may be formulated in accordance with  
7 conventional procedure by compressing mixtures of the active acetogenins and solid  
8 carriers, and lubricants well-known to those familiar with the art. Examples of solid  
9 carriers include starch, sugar, etc. The compounds of the present invention can also be  
10 administered in the form of a hard shell tablet or capsule containing, for example, lactose  
11 or mannitol as a binder, and conventional fillers and tabling agents.

12 The cytotoxicities of muricin A-G (1-7) were tested by the three days bioassay against  
13 human cancer cell lines, Hep G<sub>2</sub> and 2,2,15 according to known procedures and results are  
14 shown in Table 6. Adriamycin was used as a standard.  
15

16 Table 6. Cytotoxicity IC<sub>50</sub> values of compounds 1~12 against human hepatoma cell lines

Treatments	Human hepatoma cell lines	
	Hep G <sub>2</sub>	2,2,15
	IC <sub>50</sub> (μg/mL)	IC <sub>50</sub> (μg/mL)
Muricin A (1)	5.04	5.13 × 10 <sup>-3</sup>
Muricin B (2)	1.78	4.29 × 10 <sup>-3</sup>
Muricin C (3)	4.99 × 10 <sup>-1</sup>	3.87 × 10 <sup>-3</sup>
Muricin D (4)	6.60 × 10 <sup>-4</sup>	4.80 × 10 <sup>-2</sup>
Muricin E (5)	NT	NT

Muricin F ( <b>6</b> )	$4.28 \times 10^{-2}$	$3.86 \times 10^{-3}$
Muricin G ( <b>7</b> )	NT	NT
Muricatetrocins A&B ( <b>8&amp;9</b> )	$4.95 \times 10^{-2}$	$4.83 \times 10^{-3}$
Longifolicin ( <b>10</b> )	$4.04 \times 10^{-4}$	$4.90 \times 10^{-3}$
Corossolin ( <b>11</b> )	$3.53 \times 10^{-1}$	$2.34 \times 10^{-1}$
Corosolone ( <b>12</b> )	$4.80 \times 10^{-1}$	$2.84 \times 10^{-1}$
Adriamycin	$2.41 \times 10^{-1}$	$4.50 \times 10^{-1}$

1 NT = non-test

2  
3 According to Dr. Miyoshi's strategy, the structure-activity relationships of  
4 annonaceous acetogenins (1-12) were discussed by being dissected into four chemical  
5 portions as following: the hydroxylated THF ring moiety, the  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone  
6 ring moiety, and the spacer moiety linking the two rings, and the alkyl side chain attached  
7 to THF rings which had a diol group and ended with the terminal methyl.

8 The NMR spectral and MS data of muricin A and muricin B showed that these two  
9 annonaceous acetogenins compounds are steric isomers. For determining the absolute  
10 stereochemistry of them, their Mosher ester derivatives were prepared (see Table 2). The  
11 only difference between 1 and 2 was the stereochemistries at C-4. The very small but clear  
12 difference between (S)- and (R)-MTPA esters permitted us to conclude that the  
13 confirmations of C-4 and C-19 of muricin B should be (S) and (R), while the configuration  
14 of C-4 and C-19 of muricin A were (R) and (R). Muricin A was the first report that the  
15 configuration of the hydroxyl group at C-4 in annonaceous acetogenins could be not only R,  
16 but also S. In addition, both muricin A and muricin B are also the first examples of  
17 annonaceous acetogenins wherein the THF ring initializes at C-15. Two compounds gave

1 some space to elucidate how the orientations of the terminal lactone ring and a hydroxyl  
2 group at C-4 alter their bioactivities (see Table 6). Moreover, these two compounds were  
3 the first examples wherein the THF ring began at C-15. Although no annonaceous  
4 acetogenins with such a special position of THF ring have been reported in reviews and  
5 related papers, it should be reasonable in biosynthesis to form a THF ring in an odd  
6 position due to a close polyhydroxyl system.

7 Interestingly, muricin C is also the first example of annonaceous acetogenins wherein  
8 the THF ring began with an odd position, C-17. Muricin D and muricin E, rechecked by  
9 HRFAB-MS twice, were reported at the first time that annonaceous acetogenins possessed  
10 a C33 skeleton, while most annonaceous acetogenins were thought to possess a C37 or C35  
11 skeleton before.

12 In this invention, the relationships between bioactivity and stereochemistry of  
13 annonaceous acetogenins compounds were concluded as follows:

14 The role of the stereochemistry of the hydroxyl group at C-4:

15 Muricin A and muricin B, in which the only difference is the orientations of the  
16 hydroxyl group at C-4, revealed to the resembling bioactivities. However, it was clear that  
17 muricin A with (*R*)-hydroxyl group at C-4 was 2.5 times more potent than muricin B with  
18 (*S*)-form to against Hep G<sub>2</sub>. Although it was indicated in Miyoshi's paper that the presence  
19 of the 4-OH group in the spacer region is not essential for the activity, the stereochemical  
20 difference of the 4-OH should be the only reason why their bioactivities alter.

21 The role of the spacer moiety linking the two rings:

22 In comparison with the cytotoxic value (IC<sub>50</sub>) of muricin C and muricin D, the result  
23 revealed that, against 2,2,15, the shorter the length of the spacer moiety, the weaker the  
24 potency becomes, but, against Hep G<sub>2</sub>, the potency was not. Comparison with

1 muricatetrocin A and muricatetrocin B suggested that the appropriate length,  
2 approximately 12 carbons (from C-3 to C-14), should be more essential against Hep G<sub>2</sub>.

3 The role of hydroxyl groups in the alkyl side chain:

4 Against Hep G<sub>2</sub>, comparisons with muricin A, muricin B, and muricin D indicated  
5 that the longer the length between the THF ring and the diol group, the weaker the potency  
6 becomes.

7 The role of the double bond in the alkyl side chain:

8 For annonaceous acetogenins with the mono-THF ring with one flanking hydroxyl  
9 group, muricin F showed more cytotoxic than muricin C against either Hep G<sub>2</sub> or 2,2,15,  
10 which means that the presence of the double bond could raise the bio activity.

11 Various modifications and variations of the present invention will be recognized by  
12 those persons skilled in the art without departing from the scope and spirit of the invention.  
13 Although the invention has been described in connection with specific preferred  
14 embodiments, it should be understood that the invention as claimed should not be unduly  
15 limited to such specific embodiments. Indeed, various modifications of the described  
16 modes for carrying out the invention, which are obvious to those skilled in the art, are  
17 intended to be within the scope of the following claims.

18

19

1       Reference and Notes

2       1. Zeng, L.; Ye Q.; Oberlies, N.H.; Shi, G.; Gu, Z. M.; He, K.; McLaughlin J. L. *Nat*  
3       *Prod. Rep.* 13,275-306, 1996.

4       2. Zafra-polo, M. C.; Figadère, B.; Gallardo, T.; Tormo, J. R.; Corted, D.  
5       *Phytochemistry* 48,1089-1117, 1998.,

6       3. Alali, F. Q.; Liu, X. X.; McLaughlin, J. L. *J. Nat Prod.* 62,504-540, 1999.

7       4. Li, C. M.; Mu, Q.; Hao, X. J; Sun, H. D.; Zheng, H. L.; Wu Y. C. *Chin Chem. Lett.*  
8       5,747-750, 1994.

9       5. Reiser, M. J.; Fang, X. P.; Anderson, J. E.; Miesbauer, L. R.; Smith, D. L.;  
0       McLaughlin, J. L. *Helv. Chim. Acta*, 76,2433-2443, 1993.

1       6. Ye, Q.; alfonso, D.; Evert, D.; McLaughlin, J.L. *Bioorg. Med. Chem.* 4,537-545,  
2       1996.

3       7. Cortes, D.; Myint, S. H.; Laurens, A.; Hocquemiller, R.; Leboeuf, M.; Cavè, A.  
4       *Can J. Chem.* 69,8-11, 1991.

5       8. Riser, M. J.; Hui, Y. H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.;  
6       McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.*  
7       114,10203-10213, 1992.

8       9. Liaw, C. C.; Chang, F. R.; Chen, Y. T.; Chiu, H. F.; Wu, M. J.; Wu, Y. C.; *J. Nat.*  
9       *Prod.* 63,1613-1617, 1999.

10      10. Chang, F. R.; Chen, J. L. Lin, C. Y.; Chiu, H. F.; Wu, M. J.; Wu, Y. C.;  
11      *Phytochemistry* 51,883-889, 1999.

12      11. Chang, F. R.; Chen, Y. C.; Chiu, H. F.; Wu, M. J.; Wu, Y. C.; *Phytochemistry*  
13      51,429-33, 1999.

14      12. Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Ramirez, E. A.; Zhuang, Z.

1 tetrahedron lett. 35,8529-8532, 1994.

2 13. Chávez, D.; Mata, R. *J. Nat. Prod.* 61,580,584, 1998.

3 14. Gawronski, J.; Wu, Y. C.; Polish J. Chem. 73,241-243, 1999.

4 15. Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.;  
5 Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* 42,1175-1184, 1994.

6 16. Doong, S.; L.; Ysai, C. H.; Schinazi, R. F.; Liotta, D. C.; Cheng, Y. C. *Proc. Natl.*  
7 *Acad. Sci., USA* 88,8495-8499, 1991.

8 17. Elliott, W. M.; Auersperg, N. *Biotech. Histochem.* 68,29-35, 1993.

9 18. Miyoshi, H.; Ohshima, M.; Shimada, H.; Akagi T., Iwamura, H.; Maclughlin, J.  
0 L. *Biochim. Biophysica. Acta*, 1365,443,452. 1998.